Digest Journal of Nanomaterials and Biostructures Vol. 10, No. 2, April - June 2015, p. 655 - 662

BIOSYNTHESIS OF SILVER NANOPARTICLES USING *FUSARIUM SOLANI* AND ITS IMPACT ON GRAIN BORNE FUNGI

A. R. M. ABD EL-AZIZ[a], M. R. AL-OTHMAN[a], M. A. MAHMOUD[b], H. A. METWALY[c]
[a]*Botany and Microbiology Department, College of Science, King Saud University, Riyadh 1145, Saudi Arabia*
[b]*Plant Pathology Research Institute, Agricultural Research Center, Giza 12619, Egypt*
[c]*Central Laboratory of Organic agricultural, Agricultural Research Center, Giza 12619, Egypt*

We report extracellular biosynthesis of silver nanoparticles by *Fusarium solani* which isolated from wheat grains when challenged with 1mM silver nitrate ($AgNO_3$). The formation of nanoparticles was characterized by color change followed by UV–Vis spectrophotometric analysis, which showed a peak at about 415nm, which is very specific for silver nanoparticles. Silver nanoparticles amount were 55% showed by Energy Dispersive Spectroscopy (EDS). Transmission Electron Microscopy for particles size and morphology. In addition, in this research we study effect of silver nanoparticles on % fungal frequency at different concentrations was analyzed in PDA, in all cases, fungal frequency % were recorded zero at 4%. While no effective noticeable with treatment at 1% of AgNPs against all fungi. On the other side, data obtained that treatment with silver nanoparticles on % fungal frequencies at different concentrations were the best with compared Non-Surface sterilization or Surface sterilization with sodium hypochlorite.

(Received March 17, 2015; Accepted June 12, 2015)

Keywords: Fusarium solani, Silver nanoparticles, Biosynthesis, Characterization, Cereal grains, fungi frequencies

1. Introduction

Cereal grains constitute important sources of energy and protein. There is a significant risk of contamination with the secondary metabolites when cereal grains colonies of seed borne fungi [1, 2] which can cause enormous losses for growth and productivity of crop [3, 4].

Wheat is one of the most important products in all the world, where it can provide more than 20% of the calories of daily food intake [5]. Wheat is grown in most parts of the world. It is the most important crop among the cereal by area planted and is followed in importance by corn, barley, and sorghum. The amount of wheat traded internationally exceeds that of all other grains. Furthermore, the protein and caloric content of wheat is greater than that of any other food crop.

Corn (*Zea mays* L.) is one of the main cereals that is used as a source of food, forage, and processed products for industry. World corn production is approximately 790 million tons, and as a staple food, it provides more than one-third of the calories and protein in some countries [6].

Barley (*Hordeum vulgare* L.) like most of the economically important crops is prone to diseases. Barley is an annual cereal grain, which for serves as a major animal feed crop, barley, has been the focus of attention for many years. Yield losses due to this disease have been estimated to range from 10-40% [7] and 100% damage have also been reported [8].

Some of the seed-borne mycoflora of barley include *Alternaria alternata, Aspergillus candidus, A. flavus, Curvularia lunata, Drechslera halodes, Fusarium moniliforme, F. pallidoroseum, F. solani* and *Ulocladium* sp. [9, 10]. Seed-borne mycoflora of wheat reported recently included *Alternaria alternata, Drechslera sorokiniana, Fusarium moniliforme, F. avenaceum, F. graminearum, F. nivale, F. culmorum, F. equiseti, F. sporotirchioides,*

Cladosporium herbarum, Stemphylium botryosum [11, 12]. Survey of literature shows that a number of fungi viz., *Alternaria alternata*, *Aspergillus* spp., *Bipolaris maydis, Fusarium moniliforme*, *Fusarium* spp., *Cephalosporium* spp., *Helminthosporium* spp., *Mucor* sp., and *Penicillium* spp., have been reported from maize seed [13, 14, 15]. Plant disease cause reduced agricultural production every year which lose of millions of dollars for control these plant diseases. In recent years, using pesticide for controlling plant disease resulted many environmental hazards. Therefore many researchers try to find an alternative method for pesticides, use of silver nanoparticles as antimicrobial agents. Silver nanoparticles have many applications. Silver nanoparticles displays multiple modes of inhibitory action against microorganisms with relative safety for control of various plant pathogens, compared to synthetic fungicides [16, 17] and will lead to improved treatment strategies to control the diseases and protect production [18]. Many research have been used silver nanoparticles as antifungal like *Fusarium oxysporum, Curvularia lunata, Rhizopus arrhizus, Aspergillus niger* and *Aspergillus flavus* [19].

2. Materials and Methods

2.1 Isolation and identification of fungus

Isolation of *F. solani* was carried out from wheat, maintained on potato dextrose agar (PDA) medium at 28 ± 2C. The isolated fungus was identified on the basis of their morphological characteristics and microscopically. The identification of isolates were confirmed by Regional Center of the Fungi and their Applications, Al-Azhar University, Cairo, Egypt

2.2 Synthesis of silver nanoparticles

For the synthesis of silver nanoparticles, the biomass of fungus *F. solani* was prepared by growing the fungus aerobically in a liquid medium containing (g/l) KH_2PO_4, 7.0; K_2HPO_4, 2.0; $MgSO_4 \cdot 7H_2O$, 0.1; $(NH_2)SO_4$, 1.0; yeast extract, 0.6; and glucose, 10.0. The flasks were inoculated and then incubated on shaker at 28 C and agitated at 120 rpm for 72 h. The biomass was harvested after complete incubation by filtering through filter paper followed by repeated washing with distilled water to remove any medium component from the biomass. About 20 g (wet weight) was added to 100 ml of sterilized double distilled water for 72 h at 28 °C in a 250 mL Erlenmeyer flask and agitated again at 120 rpm. After the incubation, the cell filtrate was obtained by passing it through Whatman filter paper No. 1. The filtrate was treated with aqueous 1 mM $AgNO_3$ solution in an Erlenmeyer flask and kept on a shaker at 27 °C.

2.3 Characterization of silver nanoparticles

The characterization of silver nanoparticles was carried out by different instruments and techniques. It includes visual observation in color from yellow to brown upon completion of the reaction (Fig. 1). The formation of reduced silver nanoparticles in colloidal solution was monitored using UV-Vis spectral analysis by color changes in the supernatant Cintra 10e GBC double beam UV–Vis spectrophotometer (Victoria, Australia) at wavelengths between 200 to 800nm.

2.4 Energy Dispersive Spectroscopy (EDS)

Elemental analysis on single particles was carried out using JEOL (JSM-6380 LA).

2.5 Transmission electron microscopy

Transmission electron microscopy was performed on a JEOL (JEM-1010) instrument with an acceleration voltage of 80 kV after drying of a drop of aqueous AgNPs on a carbon-coated copper TEM grid. The particle size distribution of the silver nanoparticles was evaluated using Image 1.45s software.

2.6 Scanning electron microscopy.

The powdered particles were scanned on scanning electron microscope (SEM) using a JEOL (JSM-6380 LA) instrument

2.7 Effect of sodium hypochlorite and silver nanoparticles on fungal frequency from corn, barley and wheat:

Samples of cereal were collected from Riyadh, in the Kingdom of Saudi Arabia, were examined for seed-borne fungi. Fungi were isolated and cultured according to the method described by [20]. Five sets of each of three tested grains were used either after they were surface sterilized, without sterilization and silver nanoparticles. Surface sterilization was accomplished by immersing the seeds in 5% sodium hypochlorite solution for 5 min and followed by washing by sterile distilled water. Silver nanoparticles treatment was accomplished by soaking in with three different concentrations (1, 2 and 4%) for 2h. five pieces of each treatment were placed on the surface of Petri dishes 9 cm diam. containing potato dextrose agar (PDA), and each entry replicated three times. Petri dishes were incubated at 25 ºC and examined daily for 5 days, after which the colonies were counted. Isolates were purified either by single spore or hyphal tip methods. The identification of isolates were confirmed by Regional Center of the Fungi and their Applications, Al-Azhar University, Cairo, Egypt.

The frequency of fungi of particular species with in a genus of fungi was calculated using the formula of [21].

$$\text{Frequency} = \frac{\text{Number of fungal species isolated}}{\text{Total Number of fungi isolated}} \text{ X100}$$

3. Results and Discussion

3.1 Biosynthesis AgNPs

The synthesis of silver particles using *F. solani* was investigated [22]. After addition of $AgNO_3$ to filtered cell-free culture, the color of the mixture changed from colorless to brown with intensity increasing during the period of incubation which confirms the reduction of $AgNO_3$ by *F. solani* indicated the synthesis of AgNPs [23]. (Fig. 1) show that picture of conical flasks containing biomass of *F. solani*, the filtrate of the *F. solani* in aqueous solution of 10^{-3}M $AgNO_3$ after 3 days of reaction (Fig. 2) .Control (without silver ions) showed no change in color of the cell filtrates when incubated in the same conditions (data not shown).

Fig. 1. Conical flasks containing biomass of F. solani

Fig. 2. Filtrate of the F. solani in aqueous solution of 10^{-3}M $AgNO_3$ after 3 days of reaction

3.2 Characterization of silver nanoparticles

The formation and stability silver nanoparticles in colloidal solution was observed by using UV-Vis spectral analysis (Fig. 3). It was observed from spectra that the silver surface Plasmon resonance band occurred at 415nm. after 72 h. of incubation. The reduction of metal ions occurs on the surface by the enzymes presented in the cell wall [24, 25]. The absorptions spectra are due to Plasmon excitations of particles [26].

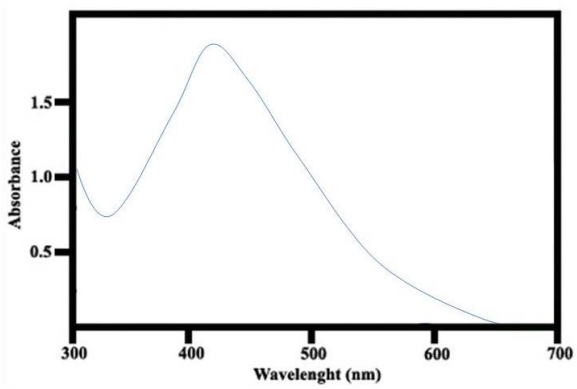

Fig. 3. The UV–Visible absorption spectra of extracellularly synthesized AgNPs by F. solani at 415 nm

The presence of elemental silver signal was confirmed by energy dispersive spectroscopy (EDS) shown in Fig. 4. The presence of an optical absorption band at ~3ev reveals the presence of pure metallic silver nanoparticles. The spectrum shows mainly Ag (55%) and other elements (45 %). [27].

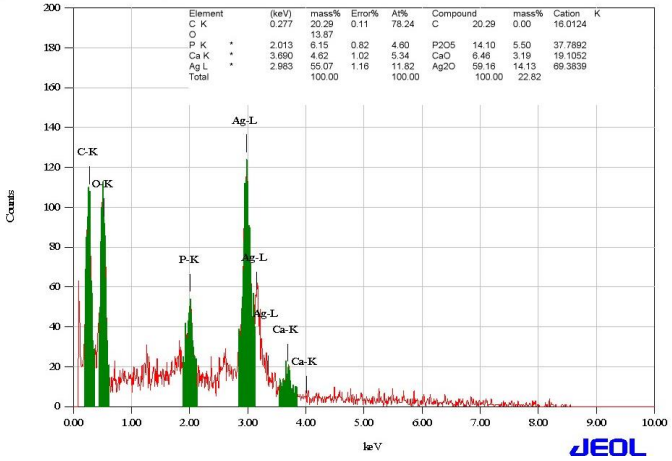

Fig. 4. EDS spectra of silver nanoparticles.

Determine the morphology and shape of nanoparticles by TEM. Fig. (5) revealed that the particles are spherical and monodispersed (shape uniformly distributed) without significant agglomeration this results are incompatible with [22] and compatible [28]. The particle size histogram (Fig. 6) shows that the particle size ranges from 5 to 30 nm. These results are compatible with [22]. The highest numbers of particles distribution are in the 10 to 15-nm range.

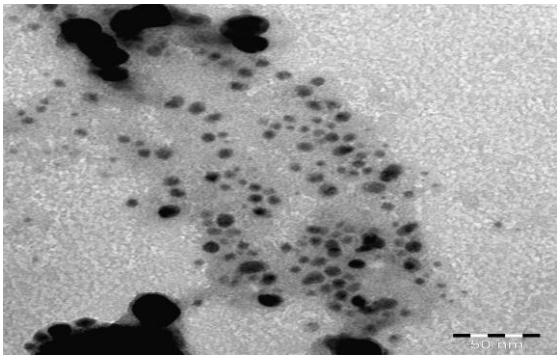

Fig. 5. Transmission Electron Microscopy (TEM) images of synthesized silver nanoparticles by F. solani.

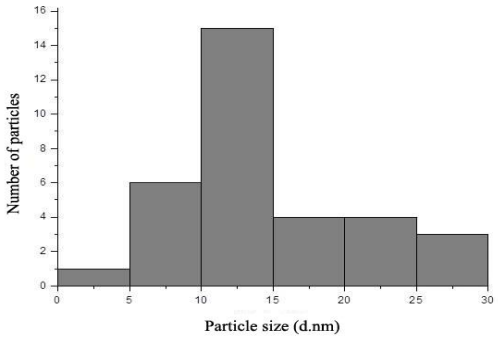

Fig. 6. A particle size distribution histogram of silver nanoparticles determined from Transmission Electron Microscopy (TEM) images

Scanning electron micrograph (Fig. 7) SEM micrograph of silver nanoparticles shows, the capping agent and the particles size can be larger than the size measured by TEM due to aggregated particles [29].

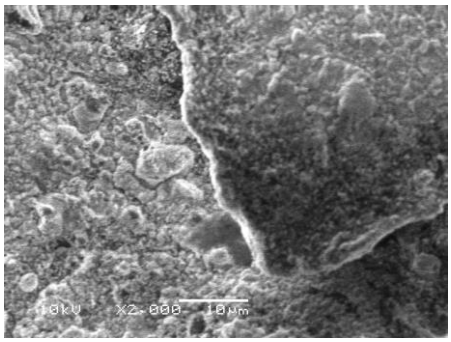

Fig. 7. Transmission Electron Microscopy (TEM) images of synthesized silver nanoparticles by F. solani

3.3 Soaking three tested cereal grains on silver nanoparticles

Data in Table (1) and Fig (8 and 9) show that effect of silver nanoparticles on % fungal frequency at different concentrations was analyzed in PDA, in all cases, fungal frequency % were recorded zero at 4%. In most cases, fungal frequency % was zero when treatment tested cereal grains with silver nanoparticles at 2%. While no effective noticeable with treatment at 1% of AgNPs against all fungi. On the other side, data obtained that treatment with silver nanoparticles on % fungal frequencies at different concentrations were the best with compared Non –Surface sterilization (Fig. 10) or Surface sterilization with sodium hypochlorite (Fig.11).

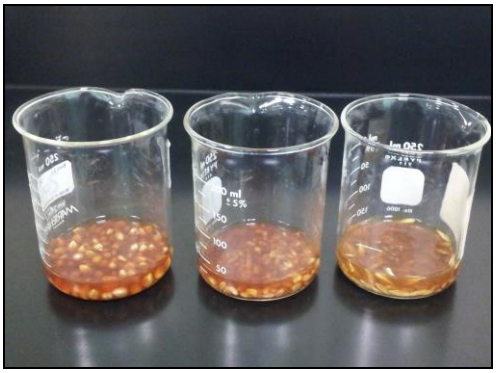

Fig. 8. Soaking three tested cereals grain in silver nanoparticles for 2h.

Table 1. Effect of silver nanoparticles on % fungal frequency were isolated from wheat, barley and corn

Fungi	Non-Surface sterilization			Surface sterilization with sodium hypochlorite			Silver nanoparticles treatments								
	wheat	barley	corn	wheat	barley	corn	wheat			barley			corn		
F. oxysporum	0.0	0.1	0.0	17.2	11.3	13.8	15.2	4.2	0.0	10.0	4.2	0.0	9.2	3.1	0.0
F. moniliform	0.3	0.0	0.4	4.4	2.6	6.2	3.0	1.8	0.0	1.4	0.5	0.0	4.3	1.1	0.0
F. solani	0.0	0.0	0.2	2.9	3.1	1.9	2.0	0.0	0.0	0.0	0.0	0.0	0.8	0.0	0.0
F. verticillioides	0.5	0.2	0.4	3.6	1.1	2.4	5.1	3.5	0.0	0.3	0.0	0.0	2.0	1.2	0.0
F. semitectum	1.1	1.4	0.7	12.6	9.2	7.5	4.5	2.8	0.0	6.1	1.7	0.0	5.7	2.8	0.0
A. flavus	8.1	6.2	5.7	6.3	4.9	12.4	5.1	3.0	0.0	3.1	0.0	0.0	4.1	1.9	0.0
A. terreus	0.0	0.3	0.1	4.6	3.4	2.8	3.1	0.0	0.0	2.3	0.0	0.0	1.4	0.3	0.0
A. niger	27.4	25.4	18.2	11.4	14.6	10.3	7.0	2.3	0.0	10.0	4.1	0.0	8.3	2.4	0.0
A. ficuum	2.4	3.1	2.0	1.2	3.1	2.8	0.6	0.2	0.0	0.4	0.0	0.0	1.0	0.0	0.0
P. citrinum	4.1	3.3	3.7	2.1	1.9	4.0	2.0	0.7	0.0	2.1	0.8	0.0	2.8	0.9	0.0
P. islandicum	3.0	2.6	1.8	0.6	1.2	0.3	0.0	0.0	0.0	1.4	0.0	0.0	0.1	0.0	0.0
P. chrysogenum	0.9	1.1	0.0	0.8	2.6	1.3	0.1	0.0	0.0	0.2	0.0	0.0	1.5	0.0	0.0
Rhizopus stolonifer	34.0	38.0	42.3	0.0	2.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Phoma	0.0	0.0	0.0	0.3	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
A. alternata	0.7	1.1	0.9	3.2	2.8	1.7	2.1	0.3	0.0	1.1	0.2	0.0	0.0	0.0	0.0
A. chlamydospora	1.15	2.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

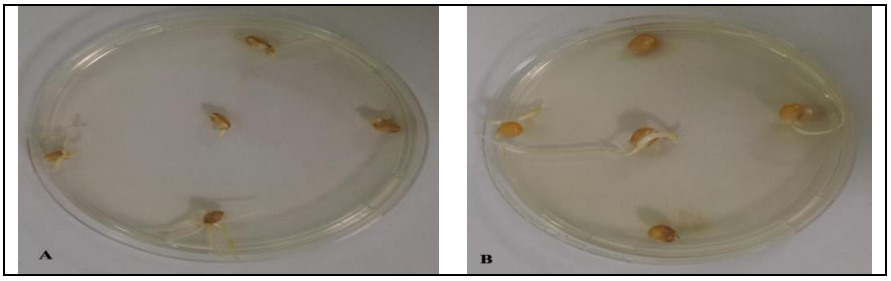

Fig. 9. Wheat (A) and corn grains (B) after soaking in silver nanoparticles at 4%concentration for 2h.

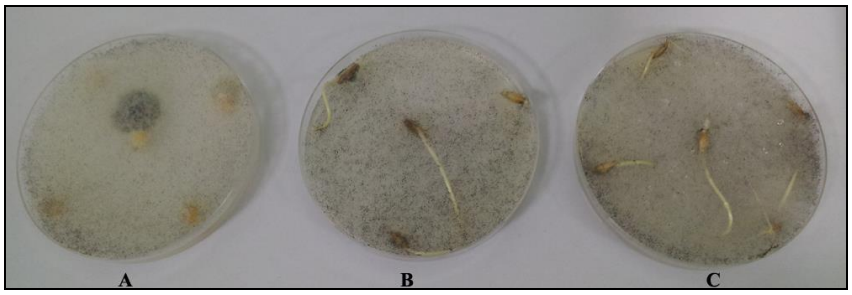

Fig. 10. Three tested cereals grains without sterilization corn (A), Barley (B) and wheat (C).

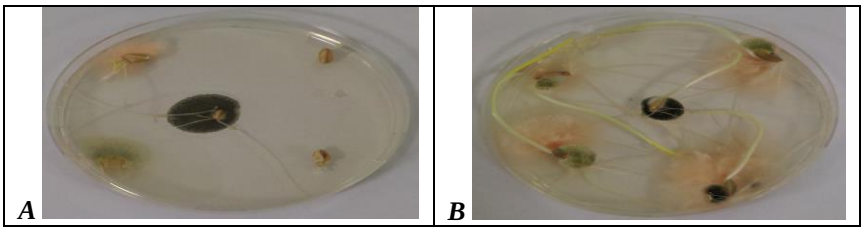

Fig. 11. Surface sterilization of wheat (A) and Barley (B) in 5% sodium hypochlorite solution for 5 min.

Silver nanoparticles have low toxicity and antifungal activity to reduce the colony formation. Also has a great potential for use in controlling spore-producing fungal plant pathogens [31]. Silver in ionic or nanoparticle forms has a high antimicrobial activity and is therefore widely used for various sterilization purposes. There have been relatively few studies on the applicability of silver to control plant diseases. Silver nanoparticles can be used effectively in the control of disease and used in control of plant diseases as antifungal and fungal alternative. These results agree with many researchers [30, 31, and 32].

4. Conclusion

We have carried out production of silver nanoparticles as safe and economically viable by successfully synthesized using culture filtrates of *F. solani* with high stability. Characterization by UV–Vis spectroscopy, TEM and EDS revealed that biosynthesized nanoparticles possess remarkable stability. Silver nanoparticles have a high antimicrobial activity and used for various sterilization, in this study data show that treatment with silver nanoparticles on % fungal frequencies at different concentrations were the best with compared Non–Surface sterilization or Surface sterilization with sodium hypochlorite.

Acknowledgments

The authors would like to extend their sincere appreciation to the Deanship of Scientific Research at king Saud University for its funding this Research group No. (RG -1435-269).

References

[1] D. Abramson, D'Mello, J.P.F. (Ed.), Handbook of Plant and Fungal Toxicants. CRC Press, Boca Raton, FL, pp. 303 (1997).
[2] J.E. Smith, In: D'Mello, J.P.F. (Ed.), Handbook of Plant and Fungal Toxicants. CRC Press, Boca Raton, FL, pp. 269 (1997).
[3] W. A. J.M. Dawson, G. L. Bateman, Plant Pathology, **50**, 5 (2001).
[4] S. M. M. Islam1, M. M. I. Masum, M. G. A. Fakir, Scientific Research and Essay. **4**, 175 (2009).
[5] M. V. Wiese. Compendium of Wheat Diseases. 2nd edn. APS Press, St. Paul (1987).
[6] S. N. Chulze, Food Addit. Contam. Part A Chem. Anal. Control. Expo. Risk Assess. **27**, 651 (2010).
[7] B. J. Steffenson, R .K. Webster, Phytopathlogy, **82**, 170 (1992).
[8] D. E. Mathre, American Phytopathological .Society, St. paul, Minnesota. (1982).
[9] M.J. Richardson, An annotated list of seed-borne diseases, Supplement 2. Int. seed Test. Assoc., Zurich, Switzerland.108 pp. (1983).
[10] S. Ahmed, S. H. Iqbal, A. N. Khalid. 1997. Fungi of Pakistan. Dept. Bot., University of the Punjab.
[11] H. H. Nirenberg, Schmitz-Elsherif, C.I. Kling, . Pflanzenkrankheiten und Pflanzenschuntz, **101**, 449 (1994).
[12] A. T. Mostafa, S. S. Kazem, S. Mohammad, M. Rokouei, Australian Journal of Basic and Applied Sciences, **5**, 1070 (2011).
[13] A. E. Desjardins. Fusarium mycotoxins Chemistry, Genetics, and Biology. St. Paul, MN: The American Phytopathological Society (2006).
[14] M. A. Mahmoud, H.M. Ali, A.R.M. El-Aziz, M.R. Al-Othman, A.S. Al-Wadai, Genetics and Molecular Research **13** , 9352 (2014).
[15] Tulin, J.M., D.I. Askun. 2006. Journal of Biological Sciences, **6**, 275 (2006).
[16] J. L. Clement, P.S. Jarrett. Met Based Drugs, **1**,467 (1994)
[17] H.J. Park, S.H. Kim, H.J. Kim, S.H. Choi, Plant Pathol J. **22**, 295 (2006).
[18] L. Cursino, Y. Li, P. A. Zaini, L. De La Fuente, , H. C. Hoch, T. J. Burr, Microbiology Letters, **299**, 193 (2009).
[19] N. Savithramma, M. Linga Rao, K. Rukmini, P. Suvarnalatha devi, International Journal of ChemTech Research , **3**,1394 (2011).
[20] A.M. Hussaini, A.G .Timothy, H.A. Olufunmilayo, A.S. Ezekiel, H.O.Godwin ,World J Agric Sci **5**, 5 (2009).
[21] S.A. Ghiasian, P. Kord-Bacheh., S.M. Rezayat, A.H. Maghsood, H. T.khani **158** , 113(2004).
[22] A. Ingle, M. Rai, A. K Gade, M.Bawskar, Journal of Nanoparticle Research **11**, 2079 (2008).
[23] K.C. Bhainsa, S. F D'Souza, Biosynthesis of colloidal-silver particles using microorganisms, Polish J. Chem, **82**, 377 (2006).
[24] P. Mukherjee, A. Ahmad, D. Mandal, S. Senapati, S. R. Sainkar, M. I. Khan, R. Ramani, R. Parischa, P. A. V. Kumar, M. Alam, M. Sastry, R. Kumar, Angew Chem. Int. **40**, 3585 (2001a).
[25] P. Mukherjee, A. Ahmad, D. Mandal, S. Senapati, R. S. Sainkar, I. M. Khan, R. Parishcha, V. P. Ajaykumar, M. Alam, R. Kumar, M. Sastry, Nano Letters **1**, 515 (2001b).
[26] C.H. Bae, S.M. Nam, S.M. Park. Applied Surface Science, **197**, 628 (2002).
[27] P. Magudapathy, P. Gangopadhyay, B. K. Panigrahi, K. G. M. Nair, S. Dhara, Phys. B. **299**, 142 (2001).
[28] B. S. Fathima, R. M. Balakrishnan, Materials Letters **132** , 428 (2014).
[29] Y.K. Jo, Plant Disease, **93**, 1037 (2009)
[30] M. A. Elamawi, Rabab, R. A. S. El-Shafey, Egypt. J. Agric. Res., **91**, 1271 (2013). Nanomedicine: Nanotechnology, Biology, and Medicine **5**, 382 (2009).
[31] M. Gajbhiye, J. Kesharwani, A. Ingle, A. Gade, M. Rai, Nanomedicine: Nanotechnology, Biology, and Medicine **5** ,382 (2009).
[32] P. Dibrov, J. Dzioba, K. K. Gosink, C.C. Hase Agents Chemother, **46**, 2668 (2002). Tech Research.**3**, 1394 (2011).